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13. ABSTRACT *(Maximum 200 Words)*

The purpose of this study is to determine whether the expression of SRC-1 family members (SRC-1, TIF2, and AIB1) is altered during breast cancer progression. The scope of the research is assessing the expression of SRC-1 family members during breast cancer progression using breast cancer tissue and adjacent normal tissue obtained from Indiana University Tissue Procurement Facility. In addition, adenovirus expressing AIB1 has been constructed for testing the functions of AIB1. We found that AIB1 protein is overexpressed in human breast cancer specimens, as compared to adjacent normal breast tissue. Comparison of AIB1 expression with tumor grading, staging and other pathological results are ongoing. This study suggests that alteration of AIB1 expression is likely to play important role in breast cancer cell proliferation.

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Introduction

The subject of the current studies is to determine the expression and functions of steroid receptor coactivator-1 (SRC-1) family members in breast cancer. The purpose of these studies is to determine whether the expression of SRC-1 family members is altered during breast cancer progression. The scope of the research is assessing the expression of SRC-1 family members during breast cancer progression using breast cancer tissue and adjacent normal tissue obtained from Indiana University Tissue Procurement Facility. We will also assess the functions of SRC-1 family members by constructing adenovirus expressing coactivators.

Body

Task 1: Completed. Collection of mammary gland samples has been completed.

Task 2: Initiated. RNA has been extracted and will be analyzed for coactivator expression using Northern blot and RT-PCR techniques.

Task 3: Eliminated per contract agreement.

Task 4: Completed. We have treated animals with estrogen benzoate or tamoxifen, and mammary gland samples have been collected for task 5 analysis of coactivator expression.

Task 5: Initiated; to examine the expression of coactivators in mammary gland upon hormone and antihormone treatment.

Task 6: We have completed the collection and embedding of human breast tumor specimens.

Task 7: We have completed the immunohistochemical staining and Western blot analysis for the expression of SRC-1 and AIB1 in human breast tumor specimens. More than 122 cases of human breast tumors have been examined for the expression of ER, PR, and p160 family members. Preliminary data are presented (see tables 1 and 2 and figures 1 and 2). SRC-1 was expressed in many cases of breast tumors. In several cases, the SRC-1 expressed was inversely correlated with the expression of ERα (Fig. 1). In addition, we found that AIB1 was overexpressed in tumors as compared to breast reduction tisssue or normal adjacent mammary epithelial cells (Fig. 2). We are facing background problems using TIF2/GRIP1 antibody for immunohistochemical staining. We are currently trying to improve this background problem.

Task 8: Tumor grading, staging, and other pathological information have been gathered (Tables 1 and 2) by the Pathologist. Statistical analysis will be performed by biostatistician Dr. Li at IUPUI.

Task 9: We have done preliminary staining of human breast cancer MCF-7 cells using anti-SRC-1 and anti-AIB1 antibodies. Nuclear staining of SRC-1 and AIB1 were observed. To a less degree, the cytoplasmic staining was observed. This is in contrast to the tumor staining we have performed. In several cases, we observed the cytoplasmic staining of SRC-1 and AIB1 in tumor cells in human breast tumors.

Task 10: Completed. To examine the expression of p160 coactivators in human breast cancer cell lines, we have done Western blot analysis for SRC-1, GRIP1/TIF2, and AIB1 in several human breast cancer cell lines. Both ERα-positive (MCF-7, T47D, and ZR-75-1) and ERα-negative (HCC1937, MCF10A, MDA-MB-231, MDA-MB-435S, BT-20, and SKBR) cells express all three coactivators. MCF-7 cells expressed a much higher level of AIB1 among all the cell lines tested.

Task 11: We have successfully constructed the sense construct of the adenovirus expressing AIB1. We also tagged the virus with GFP in order to monitor the infection and expression efficiency of this virus. The control virus AdGFP has also been constructed. Preliminary data indicate that overexpression of AIB1 increased the S phase of cell cycle. The AdGFP-AIB1 will allow us to examine its roles in modulating ER signaling in situ in mammary gland. The antisense AIB1 construct is still under construction.

Task 12: We have completed testing adenoviruses constructed in tissue culture cells for this task. We have performed Western blot analysis and are able to show that AdGFP-AIB1 express functional AIB1 (Fig. 3).

Task 13: Large scale preparation of adenoviruses has been completed through CsCl banding.

Task 14: We have initiated this task and will infuse the purified adenoviruses into rat mammary gland for testing the effects of AIB1 on modulating ER signaling pathway in situ.

Task 15: As soon as the task 14 is completed, we will be able to collect samples for histology examination and in situ β-galactosidase reporter activity in order to complete this task.

Key Research Accomplishments

-We have demonstrated that AIB1 protein was altered in many breast cancer tumors.

- We have demonstrated that AIB1 over-expression could increase the S phase of the cell cycle.

-We have successfully constructed adenovirus expressing AIB1 for further testing of AIB1 function in mammary gland.

Reportable Outcomes

Abstract:

1 .Zhang, Q.-H., Chang, L.-Y., Vieth, E., Stallcup, M.R., Edwards, D.P., Cheng, L., Goulet, R.J., and Jeng, M.-H. Over-expression of Several Nuclear Receptor Coactivator Proteins in Human Breast Carcinoma. 83rd Annual Meeting of the Endocrine Society, June, 2001.

Conclusions

During this funding period, we have completed many of the tasks, especially for the analysis of p160 coactivator expression in human breast tumors. Statistical analysis is currently been conducted by biostatistician at IUPUI. Once these analyses are available, a manuscript will be prepared for publication to document the findings. In addition, the AdGFP-AIB1 that is generated during this grant period will provide a valuable tool to further assess the functional role of AIB1 in

ER transactivation function in mammary gland and in regulating breast cancer cell proliferation. Our studies suggest that overexpression of AIB1 protein may contribute to increased S phase and subsequently increased cell proliferation in human breast epithelial cells.

References

1. Shim, W.-S., DiRenzo, J., DeCaprio, J. A., Santen, R. J., Brown, M. J., Jeng, M.-H. Segregation of steroid receptor coactivator-1 from steroid receptors in mammary epithelium. Proceedings of the National Academy of Sciences 96(1): 208-213, 1999.

2. Jeng, M.-H., Kao, C., Sivaraman, L., Krnacik, S., Chung, L. W. K., Medina, D., Conneely, O. M., and O'Malley, B. W. Reconstitution of estrogen dependent transcriptional activation of an adenoviral target gene in select regions of the rat mammary gland. Endocrinology 139(6): 2916-2925, 1998.

3 .Zhang, Q.-H., Chang, L.-Y., Vieth, E., Stallcup, M.R., Edwards, D.P., Cheng, L., Goulet, R.J., and Jeng, M.-H. Over-expression of Several Nuclear Receptor Coactivator Proteins in Human Breast Carcinoma. 83rd Annual Meeting of the Endocrine Society, June, 2001.

Appendices

Three figures and two tables.

Table 1

Clinicopathological factors of the breast carcinoma patients

Factor	Number	Percentage (%)
Age		
< 50	39	31.97
>=50	83	68.03
Tumor size		
< 2cm	57	53.27
2.1-5cm	44	41.12
> 5cm	6	5.61
Lymph node		
Negative	56	53.85
Positive	48	46.15
Staging		
1	35	35.00
2	54	54.00
3	11	11.00
Grading		
Well	11	10.68
Moderate	49	47.57
Poor	43	41.75
ER status		
Negative	36	29.51
Positive	86	70.49
PR status		
Negative	71	58.20
Positive	51	41.80

Table.2

AIB1 Immunoactivity of Normal Breast tissue and Breast Carcinoma

		AIB1 Staining Intensity			
	n	0	1	2	3
breast reduction tissue	9	1	6	2	0
adjacent Breast tissue	105	37	50	17	1
carcinoma	122	18	53	34	17

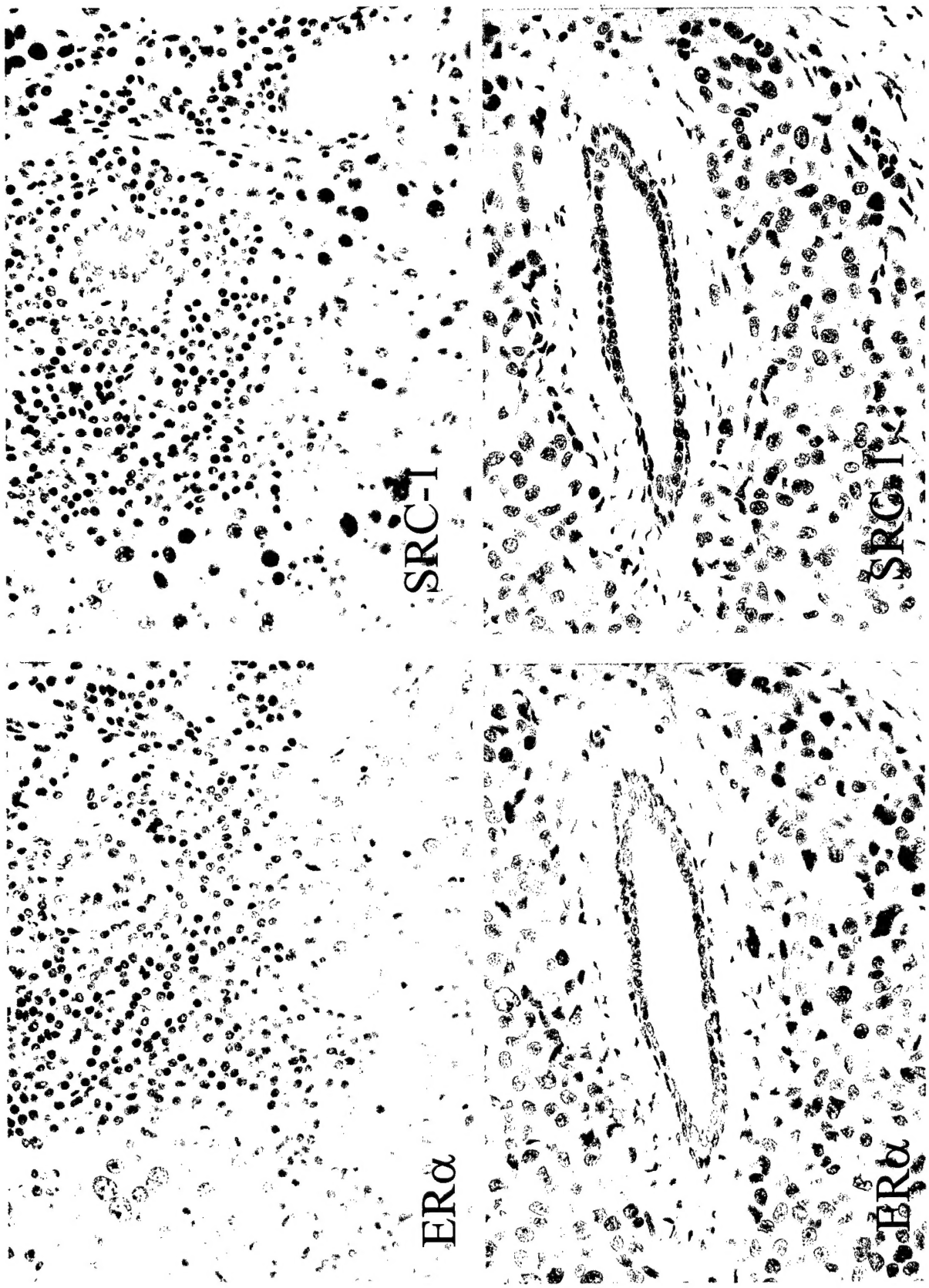

Fig. 1 Immunohistochemical staining of ERα and SRC-1 in human breast tumors. Brown color indicate cells staining positive for either ERα or SRC-1.

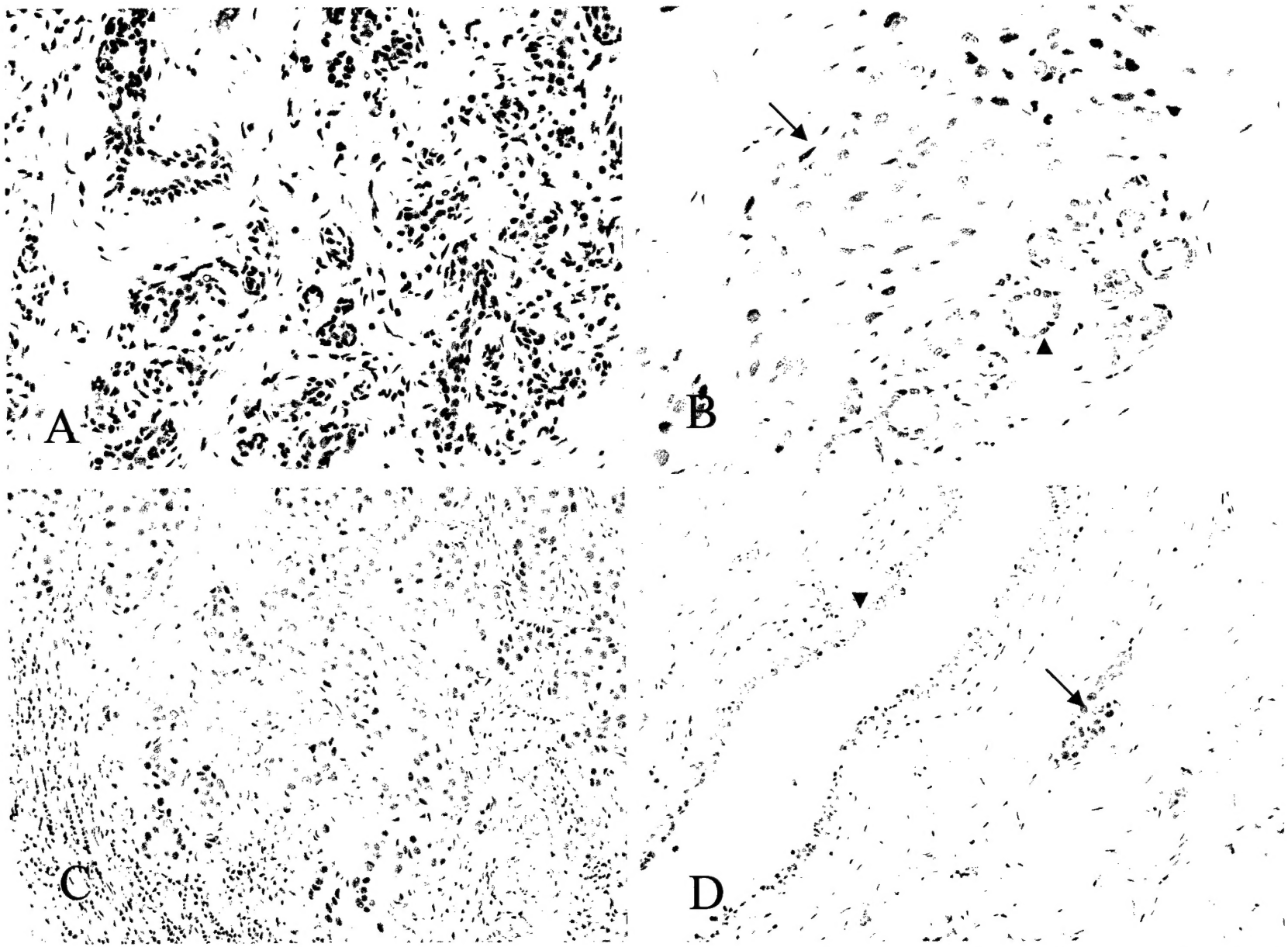

Fig.2 Immunohistochemical staining of AIB1 in human breast reduction tissue (A) and breast tumors (B-D) using anti-AIB1 antibody. Arrow heads indicate the normal epithelial cells and arrows indicate the tumor cells. Brown color indicates the cells stained positive for AIB1.

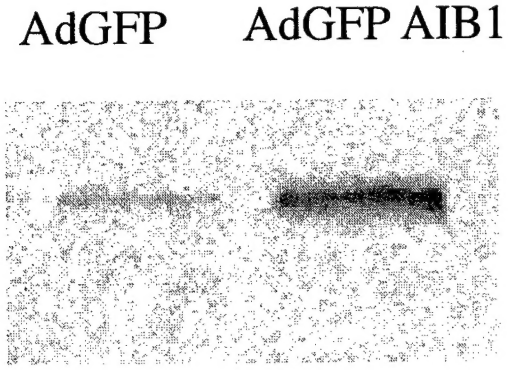

Fig. 3 Western blot analysis of AIB1 expression in MDA-MB-231 cells infected with either AdGFP or AdGFP AIB1 using anti-AIB1 antibody.